REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 10 μm Chiralcel OJ
Mobile phase: MeOH
Flow rate: 0.5
Injection volume: 20
Detector: UV 210

CHROMATOGRAM
Retention time: 10 (R-(+)), 16 (S-(-))

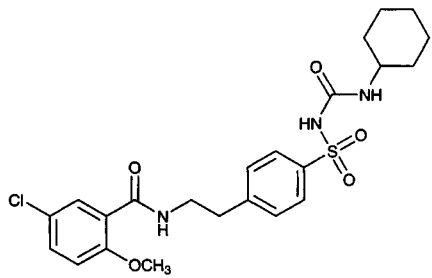
OTHER SUBSTANCES
Simultaneous: metabolites, 5-hydroxyglutethimide

KEY WORDS
chiral

REFERENCE
Weinz,C.; Blaschke,G.; Schiebel,H.-M. Investigation of the stereoselective in vitro biotransformation of glutethimide by high-performance liquid chromatography and capillary electrophoresis, *J.Chromatogr.B*, **1997**, *690*, 233–242.

Glyburide

Molecular formula: $C_{23}H_{28}ClN_3O_5S$
Molecular weight: 494.01
CAS Registry No.: 10238-21-8
Merck Index: 4486
Lednicer No.: 2 139



SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 229

CHROMATOGRAM
Retention time: 6.25
Limit of detection: <120 ng/mL

KEY WORDS
whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE
Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 21.953

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

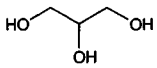
OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine,

puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Glycerin



Molecular formula: $C_3H_8O_3$
Molecular weight: 92.09
CAS Registry No.: 56-81-5
Merck Index: 4493

SAMPLE
Matrix: bulk
Sample preparation: Prepare an aqueous solution, inject an aliquot.

HPLC VARIABLES
Guard column: 10 μm Guard-pak (Waters)
Column: 250 × 4.6 5 μm Ultrasphere ODS C18
Mobile phase: MeCN:water 5:95
Flow rate: 1
Detector: RI

REFERENCE

Cannon,J.M.; Brown,R.D.; Murrill,E.M.; Jameson,C.W. Identification of components in iodinated glycerol, *J.Pharm.Sci.*, **1989**, *78*, 48–51.

SAMPLE
Matrix: bulk
Sample preparation: Prepare an aqueous solution, inject an aliquot.

HPLC VARIABLES
Guard column: 10 μm Guard-pak (Waters)
Column: 250 × 4.6 5 μm Ultrasphere ODS C18
Mobile phase: MeCN:water 5:95
Flow rate: 1
Detector: RI

REFERENCE

Viñas,P.; López Erroz,C.; Hernández Canals,A.; Hernández Córdoba,M. Liquid chromatographic analysis of sulfonamides in foods, *Chromatographia*, **1995**, *40*, 382–386.

SAMPLE
Matrix: formulations
Sample preparation: Remove the water from 10 μL syrup under reduced pressure for 10 min, reconstitute with 2 mL pyridine. Remove a 25 μL aliquot and add it to 75 μL reagent, shake well, let stand at room temperature for 10 min, evaporate to dryness under reduced pressure

at room temperature, flush the tube with a stream of air or nitrogen, add 2 mL 5% sodium carbonate solution containing 2.5 mg/mL 4-dimethylaminopyridine, shake or sonicate for 5 min, extract with 2 mL chloroform. Wash the extract with 2 mL 5% sodium bicarbonate solution, wash twice with 3 mL portions of 50 mM HCl containing 5% NaCl, inject an aliquot. (Prepare reagent by dissolving 100 mg 4-nitrobenzoyl chloride in pyridine with gentle warming.)

HPLC VARIABLES
Column: 150 × 3 5 μm LiChrosorb SI 60
Mobile phase: n-Hexane:chloroform:MeCN 10:3:1.9 containing 0.1% water
Flow rate: 1.4
Injection volume: 50
Detector: UV 260

CHROMATOGRAM
Retention time: 2.5

OTHER SUBSTANCES
Simultaneous: dextrose, fructose, propylene glycol, saccharose, sorbitol

KEY WORDS
syrup; derivatization; normal phase

REFERENCE
Nachtmann,F.; Budna,K.W. Sensitive determination of derivatized carbohydrates by high-performance liquid chromatography, *J.Chromatogr.*, **1977**, *136*, 279–287.

SAMPLE
Matrix: formulations
Sample preparation: Condition a C18 Sep-Pak SPE cartridge with 2 mL MeOH and 20 mL water. Dilute formulation ten-fold with water, add a 0.5 mL aliquot to the SPE cartridge, elute with three 1 mL portions of water, inject a 10 μL aliquot of the eluate.

HPLC VARIABLES
Column: 8 mm i.d. C18 radial compression (Waters)
Mobile phase: Water
Flow rate: 1
Injection volume: 10
Detector: RI

CHROMATOGRAM
Retention time: 3.4

OTHER SUBSTANCES
Simultaneous: dihydroxyacetone, dioxane, ethylene glycol, formic acid, glyceraldehyde, methylglyoxal, propylene glycol

KEY WORDS
SPE

REFERENCE
Bobin,M.F.; Martini,M.C.; Gudefin,A.; Cotte,J. Dosage de la dihydroxyacétone dans les émulsions [Assay of dihydroxyacetone in emulsions], *Farmaco.[Prat].*, **1983**, *38*, 403–414.

SAMPLE
Matrix: formulations
Sample preparation: Weigh out 1-2 mL, add 20 mL water, mix thoroughly, make up to 100 mL with water, mix thoroughly, inject a 25 μL aliquot.

HPLC VARIABLES
Guard column: Micro-Guard ion exclusion cartridge (Bio-Rad)

Column: 300 × 7.8 Aminex HPX-87H (Bio-Rad)
Mobile phase: 6.5 mM sulfuric acid
Column temperature: 65
Flow rate: 0.8
Injection volume: 25
Detector: RI

CHROMATOGRAM
Retention time: 11.62

REFERENCE
Del Grosso,A.V.; May,J.C. Gas chromatographic, liquid chromatographic, and titrimetric procedures for determination of glycerin in allergenic extracts and diagnostic antigens: comparative study, *J.Assoc.Off. Anal.Chem.*, **1987**, *70*, 825–828.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 10 μL aliquot of an aqueous solution.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC-NH_2
Mobile phase: MeCN:water 75:25
Column temperature: 22
Flow rate: 1
Injection volume: 10
Detector: RI

CHROMATOGRAM
Retention time: 5

OTHER SUBSTANCES
Simultaneous: dextrose, fructose, maltose, sucrose

REFERENCE
Johnson,J.M.; Harris,C.H. Selecting the most effective filtration media for HPLC analysis of saccharides, *J.Chromatogr.Sci.*, **1987**, *25*, 267–269.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: ION-300
Mobile phase: 2.5 mM sulfuric acid
Column temperature: 70
Flow rate: 0.4
Detector: RI

CHROMATOGRAM
Retention time: 24

OTHER SUBSTANCES
Simultaneous: acetic acid, citric acid, dextrose, EtOH, fructose, lactic acid, malic acid, MeOH, tartaric acid

REFERENCE
Keystone Scientific Catalog, 1993-4, p. 45.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: Hamilton PRP-X300
Mobile phase: MeCN:0.5 mM sulfuric acid 10:90
Flow rate: 3
Injection volume: 1
Detector: RI

OTHER SUBSTANCES
Simultaneous: i-butanol, n-butanol, s-butanol, t-butanol, EtOH, isopropanol, MeOH, n-propanol

REFERENCE
Keystone Scientific Catalog, 1993-4, p. 23.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: Shodex Sugar SP 0810P and SP 0810
Mobile phase: water
Column temperature: 80
Flow rate: 0.5
Detector: RI

CHROMATOGRAM
Retention time: 27

OTHER SUBSTANCES
Simultaneous: arabinose, dextrose, fructose, galactose, lactose, lactulose, mannitol, pullulan P-10, raffinose, sorbitol, stachyose, sucrose, xylitol

REFERENCE
Majors,R.E. Polymeric liquid chromatography column technology in Japan, *LC.GC*, **1993**, *11*, 778–788.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 100 × 7.8 Sarasep AL-1 (Sarasep, MetaChem)
Mobile phase: 50 mg/L dicalcium EDTA
Column temperature: 85
Flow rate: 0.7
Detector: RI

CHROMATOGRAM
Retention time: 2

OTHER SUBSTANCES
Simultaneous: EtOH, isoamyl alcohol, n-amyl alcohol

REFERENCE
MetaChem Catalog, **1994**, p. 65.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: RSpak DC-613 (Shodex, Phenomenex)
Mobile phase: MeCN:water 80:20
Flow rate: 1

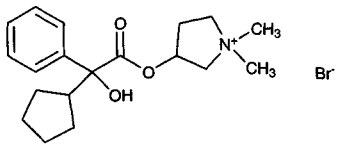
Detector: RI

CHROMATOGRAM
Retention time: 4.5

OTHER SUBSTANCES
Simultaneous: meso-erythrite, arabite, xylite, mannite, sorbite

REFERENCE
Phenomenex Catalog, **1994**, p. 1.109.

Glycopyrrolate



Molecular formula: $C_{19}H_{28}BrNO_3$
Molecular weight: 398.34
CAS Registry No.: 596-51-0
Merck Index: 4511

SAMPLE
Matrix: formulations
Sample preparation: Inject a 20 μL aliquot.

HPLC VARIABLES
Column: 100 × 4.6 Spheri-5 RP-8
Mobile phase: MeCN:buffer 45:55 (Buffer was 10 mM KH_2PO_4 adjusted to pH 4.0 with 1 M KOH.)
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 11.0
Limit of detection: 6.9 μg/mL

OTHER SUBSTANCES
Simultaneous: ondansetron
Noninterfering: degradation products

KEY WORDS
injections; saline

REFERENCE
Venkateshwaran,T.G.; King,D.T.; Stewart,J.T. HPLC determination of ondansetron-atropine and ondansetron-glycopyrrolate mixtures in 0.9% sodium chloride injection, *J.Liq.Chromatogr.*, **1995**, *18*, 2647–2659.

SAMPLE
Matrix: urine
Sample preparation: Condition a 500 mg 14 mL 40 μm CCX-2 cation-exchange SPE cartridge (Worldwide Monitoring) with two 2.5 mL aliquots of MeOH, two 2.5 mL aliquots of water, and two 2.5 mL aliquots of 100 mM pH 7.00 phosphate buffer, do not allow to dry. 5 mL Urine + 3 mL 100 mM pH 7.00 phosphate buffer + 12.5 ng mepenzolate + 5 mL water, centrifuge at 800 g for 5 min, add to the SPE cartridge, wash with 5 mL MeOH, wash with 5 mL water, dry under vacuum for 5 min, elute with 4 mL MeOH:500 mM pH 3.00 ammonium acetate 95:5 (all flow rates were 1-2 mL/min). Evaporate the eluate under a stream of nitrogen at 60°, reconstitute in 100 μL MeOH, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.1 10 μm LiChroma (Chromatographic Specialties)
Mobile phase: MeOH:50 mM pH 3.0 ammonium acetate 80:20
Flow rate: 0.8
Injection volume: 10
Detector: MS, Sciex API III triple quadrupole, ion spray interface, split column effluent 95:5 before entering detector, nebulizing gas air at 550 kPa, collision gas argon, curtain gas nitrogen, positive-ion mode, m/z 318 and 116

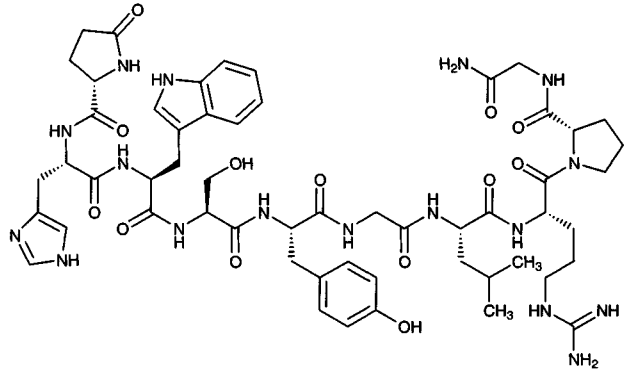
CHROMATOGRAM
Retention time: 2.3
Internal standard: mepenzolate (m/z 340 and 130) (2.1)
Limit of detection: 0.25 ng/mL

KEY WORDS
SPE; horse

REFERENCE
Matassa,L.C.; Woodard,D.; Leavitt,R.K.; Firby,P.; Beaumier,P. Solid-phase extraction techniques for the determination of glycopyrrolate from equine urine by liquid chromatography-tandem mass spectrometry and gas chromatography-mass spectrometry, *J.Chromatogr.*, **1992**, *573*, 43–48.

Gonadorelin

Molecular formula: $C_{55}H_{75}N_{17}O_{13}$
Molecular weight: 1182.31
CAS Registry No.: 33515-09-2, 51952-41-1 (HCl), 52699-48-6 (sulfate)
Merck Index: 5500



SAMPLE
Matrix: blood
Sample preparation: Condition a 1 mL Analytichem weak cation-exchange (carboxymethylhydrogen form, CBA) SPE cartridge with 1 mL 1% trifluoroacetic acid in MeOH, 1 mL MeOH, and 2 mL water. Add 1 mL plasma to the SPE cartridge, rinse the tube with 1 mL water, add the rinse to the SPE cartridge, wash with 1 mL 1% trifluoroacetic acid in water, wash with 2 mL water, wash with 2 mL MeOH, elute with 2 mL 1% trifluoroacetic acid in MeOH. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 100 μL MeOH:buffer 50:50, inject a 5-75 μL aliquot. (Buffer was 5.7 g monochloroacetic acid, 2.0 g NaOH, and 0.2 g disodium EDTA in 1 L water, pH 3.2.) [Procedure was not necessarily validated for this compound.]; SPE

HPLC VARIABLES
Column: 250 × 2 5 μm Ultrasphere octyl
Mobile phase: Gradient. A was MeOH containing 10 mM sodium octanesulfonate. B was buffer containing 10 mM sodium octanesulfonate. A:B from 45:55 to 70:30 over 30 min, maintain at 70:30 for 1 h. (Buffer was 5.7 g monochloroacetic acid, 2.0 g NaOH, and 0.2 g disodium EDTA in 1 L water, pH 3.2.)
Column temperature: 60
Flow rate: 0.3
Injection volume: 5-75

Detector: F ex 390 em 470 following post-column reaction. The column effluent mixed with 400 mM NaOH pumped at 0.15 mL/min and 0.05% ninhydrin pumped at 0.05 mL/min and the mixture flowed through a 12 m × 0.33 mm i.d. reaction coil at 70° to the detector.

CHROMATOGRAM
Retention time: 22
Limit of detection: 100 fmole

OTHER SUBSTANCES
Simultaneous: adrenocorticotropin, angiotensin I, angiotensin II, angiotensin III, atrial natriuretic peptide, bombesin, bradykinin, somatoliberin, vasopressin

KEY WORDS
plasma; SPE; post-column reaction

REFERENCE
Rhodes,G.R.; Boppana,V.K. High-performance liquid chromatographic analysis of arginine-containing peptides in biological fluids by means of a selective post-column reaction with fluorescence detection, *J.Chromatogr.*, **1988**, *444*, 123–131.

SAMPLE
Matrix: formulations

HPLC VARIABLES
Column: 100 × 5 Nucleosil 5 C8
Mobile phase: MeOH:buffer 23:77 (Buffer was 100 mM phosphoric acid adjusted to pH 3.0 with triethylamine.)
Flow rate: 1.5
Detector: UV 220

CHROMATOGRAM
Retention time: 23

OTHER SUBSTANCES
Simultaneous: impurities

REFERENCE
Storring,P.L.; Corran,P.H.; Gaines Das,R.E.; Calam,D.H. The International Reference Preparation of Gonadorelin for Bioassay: a comparison with different preparations of synthetic luteinizing hormone releasing hormone using physicochemical methods of analysis, different bioassays and immunoassay, *J.Endocrinol.*, **1982**, *95*, 95–103.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 4.6 Zorbax ODS
Mobile phase: MeCN:water:pH 3.0 triethylamine phosphate buffer 14:43:43
Flow rate: 1.5
Detector: UV 210

CHROMATOGRAM
Limit of detection: 100 ng/mL

KEY WORDS
validation

REFERENCE
Bi,M.; Singh,J. Modified HPLC method for quantification of luteinizing hormone-releasing hormone (Abstract 3367), *Pharm.Res.*, **1997**, *14*, S585.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 125 × 4 5 μm LiChrospher 100 RP-18
Mobile phase: MeCN:0.1% trifluoroacetic acid 16:84
Flow rate: 1
Injection volume: 20
Detector: UV 214

CHROMATOGRAM
Retention time: 10

OTHER SUBSTANCES
Simultaneous: degradation products

REFERENCE
Hoitink,M.A.; Beijnen,J.H.; Bult,A.; van der Houwen,O.A.G.J.; Nijholt,J.; Underberg,W.J.M. Degradation kinetics of gonadorelin in aqueous solution, *J.Pharm.Sci.*, **1996**, *85*, 1053–1059.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 125 × 4 5 μm Lichrosphere 100 RP-18
Mobile phase: MeCN:0.1% trifluoroacetic acid 16:84
Flow rate: 1
Detector: UV 214

OTHER SUBSTANCES
Simultaneous: degradation products

REFERENCE
Hoitink,M.A.; Beijnen,J.H.; Boschma,M.U.S.; Bult,A.; Hop,E.; Nijholt,J.; Versluis,C.; Wiese,G.; Underberg,W.J.M. Identification of the degradation products of gonadorelin and three analogues in aqueous solution, *Anal.Chem.*, **1997**, *69*, 4972–4978.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: Gradient. A was 0.1% phosphoric acid. B was MeCN:0.1% phosphoric acid 70:30. A:B from 95:5 to 30:70 over 20 min.
Flow rate: 1
Detector: UV 206 or RIA

CHROMATOGRAM
Retention time: 17

OTHER SUBSTANCES
Simultaneous: protirelin, somatostatin, substance P

REFERENCE
McDermott,J.R.; Smith,A.I.; Biggins,J.A.; Al-Noaemi,M.C.; Edwardson,J.A. Characterization and determination of neuropeptides by high-performance liquid chromatography and radioimmunoassay, *J.Chromatogr.*, **1981**, *222*, 371–379.

SAMPLE
Matrix: solutions

Sample preparation: Cool in ice while mixing 200 μL solution, 100 μL 4 mM benzoin in 2-methoxyethanol, 100 μL mercaptoethanol solution, and 200 μL 2 M KOH, heat on a boiling water bath for 5 min, cool in ice-water for 2 min, add 200 μL 4 M HCl:buffer 50:50, inject a 100 μL aliquot. (Prepare mercaptoethanol solution by dissolving 780 mg β-mercaptoethanol and 2.52 g sodium sulfite in 80 mL water, make up to 100 mL with water. Prepare buffer by dissolving 12.11 g Tris in 80 mL water, adjusting pH to 9.2 with concentrated HCl, and making up to 100 mL with water.)

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak phenyl
Mobile phase: Gradient. MeOH:water:500 mM pH 8.5 Tris-HCl buffer 50:35:15 for 2 min, to 80:5:15 over 24 min, maintain at 80:5:15 for 2 min.
Flow rate: 0.8
Injection volume: 100
Detector: F ex 325 em 425

CHROMATOGRAM

Retention time: 14.5-18.5

OTHER SUBSTANCES

Simultaneous: N-α-acetylarginine, agmatine, angiotensin I, angiotensin II, angiotensin III, arginine, argininosuccinic acid, bradykinin, canavanine, creatine, creatinine, guanidine, guanidinoacetic acid, guanidinobutyric acid, guanidinopropionic acid, guanidinosuccinic acid, homoarginine, methylguanidine, neurotensin, phenylguanidine, taurocyamine, tuftsin

KEY WORDS

derivatization

REFERENCE

Kai,M.; Miyazaki,T.; Yamaguchi,M.; Ohkura,Y. High-performance liquid chromatography of guanidino compounds using benzoin as a pre-column fluorescent derivatization reagent, *J.Chromatogr.*, **1983**, *268*, 417–424.

SAMPLE

Matrix: solutions
Sample preparation: Cool in ice while mixing 100 μL of an aqueous solution, 50 μL 5 mM benzoin in 2-methoxyethanol, 50 μL mercaptoethanol solution, and 100 μL 0.8 M KOH, heat on a boiling water bath for 1.5 min, add 100 μL 1.6 M HCl:1 M pH 8.5 Tris-HCl buffer 50:50, inject a 100 μL aliquot. (Prepare mercaptoethanol solution by dissolving 780 mg β-mercaptoethanol and 2.52 g sodium sulfite in 80 mL water, make up to 100 mL with water.)

HPLC VARIABLES

Column: 15 × 4 (sic) 5 μm LiChrosorb RP-18
Mobile phase: MeCN:50 mM pH 8.5 phosphate buffer 31:69
Flow rate: 0.8
Injection volume: 100
Detector: F ex 325 em 425

CHROMATOGRAM

Retention time: 22

OTHER SUBSTANCES

Simultaneous: angiotensin I, angiotensin II, angiotensin III, leupeptin acid, substance P, tuftsin

KEY WORDS

derivatization

REFERENCE

Kai,M.; Miyazaki,T.; Sakamoto,Y.; Ohkura,Y. Use of benzoin as pre-column fluorescence derivatization reagent for the high-performance liquid chromatography of angiotensins, *J.Chromatogr.*, **1985**, *322*, 473–477.

SAMPLE
Matrix: solutions
Sample preparation: Inject an aliquot of a solution in saline or Sorensen's buffer.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Econosphere endcapped C18
Mobile phase: MeCN:water 80:20 containing 0.1% trifluoroacetic acid
Detector: UV 278

CHROMATOGRAM
Retention time: 5.2

OTHER SUBSTANCES
Simultaneous: impurities

REFERENCE
Miller,L.L.; Kolaskie,C.J.; Smith,G.A.; Rivier,J. Transdermal iontophoresis of gonadotropin releasing hormone (LHRH) and two analogues, *J.Pharm.Sci.*, **1990**, *79*, 490–493.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 TSKgel ODS-120T
Mobile phase: Gradient. A was MeOH:water 20:80 containing 0.05% trifluoroacetic acid. B was MeOH:water 50:50 containing 0.05% trifluoroacetic acid. A:B from 100:0 to 0:100 over 1 h.
Flow rate: 1
Detector: UV 220

CHROMATOGRAM
Retention time: 9

OTHER SUBSTANCES
Simultaneous: angiotensin I, angiotensin II, α-endorphin, β-endorphin, calcitonin (human), protirelin (TRH), somatostatin

REFERENCE
Varian Catalog, **1993**, p. 182.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 200 × 3 Spherisorb S5ODS-2
Mobile phase: Gradient. A was 0.05% phosphoric acid containing 0.5% $(NH_4)_2SO_4$. B was MeCN. A:B from 82:18 to 64:36 over 25 min, maintain at 64:36 for 2.5 min, return to initial conditions over 1 min, re-equilibrate for 6.5 min. or Isocratic MeCN:0.05% phosphoric acid containing 0.5% $(NH_4)_2SO_4$ 24:76
Flow rate: 0.5
Detector: UV 210

CHROMATOGRAM
Retention time: 9 (gradient), 2.5 (isocratic)

OTHER SUBSTANCES
Simultaneous: buserelin, deslorelin, goserelin, leuprolide, nafarelin

KEY WORDS
comparison with capillary electrophoresis

REFERENCE

Corran,P.H.; Sutcliffe,N. Identification of gonadorelin (LHRH) derivatives: comparison of reversed-phase high-performance liquid chromatography and micellar electrokinetic chromatography, *J.Chromatogr.*, **1993**, *636*, 87–94.

SAMPLE

Matrix: tissue

Sample preparation: Condition a Bond Elut C18 SPE cartridge with 3 mL water and 3 mL MeCN (in this order ?). Homogenize 400 mg brain tissue with 2 mL 100 mM HCl, add 20 μL 10 μM IS, add 2 mL acetone, mix, centrifuge at 2450 g for 15 min. Remove the supernatant and add it to 220 μL 1 M sodium bicarbonate and 500 μL 100 mM disodium EDTA, centrifuge at 2450 g for 15 min. Remove the supernatant and evaporate it to remove the acetone, dilute the aqueous residue with 2 mL water, add to the SPE cartridge. wash with 1 mL water, wash with 3 mL 100 mM HCl, wash with two 3 mL portions of dichloromethane, wash with 1 mL water, wash with 2 mL 100 mM pH 8.0 phosphate buffer, wash with 2 mL water, elute with 2 mL MeCN:100 mM pH 2.3 phosphate buffer 70:30. Evaporate the eluate under reduced pressure, make up to 400 μL with water, inject a 100 μL aliquot.

HPLC VARIABLES

Column: 200 × 4 5 μm TSKgel ODS-120T (Tosoh)

Mobile phase: Gradient. A was MeCN:300 mM pH 2.3 sodium phosphate buffer:water 1:20:79. B was MeCN:300 mM pH 2.3 sodium phosphate buffer:water 60:20:20. A:B from 90:10 to 55:45 over 33 min, maintain at 55:45 for 7 min, to 0:100 (step gradient), maintain at 0:100.

Flow rate: 1

Injection volume: 100

Detector: F ex 325 em 435 following post-column reaction. The column effluent mixed with 2 mM benzoin in 1.6 M KOH containing 700 mM 2-mercaptoethanol and this mixture flowed through a 15 m × 0.33 mm ID PTFE coil at 76 ± 1°. The effluent from this coil mixed with 500 mM Tris containing 2.1 M HCl pumped at 0.4 mL/min and this mixture flowed to the detector.

CHROMATOGRAM

Retention time: 29.2

Internal standard: [D-Phe^{11}]-neurotensin (40.0)

Limit of detection: 0.5 pmole

OTHER SUBSTANCES

Extracted: bradykinin, dynorphin 1-8, kallidin, leucine enkephalin-Arg, methionine enkephalin-Arg-Gly-Leu, methionine enkephalin-Arg-Phe, α-neoendorphin, β-neoendorphin, neurotensin, substance P, vasopressin

KEY WORDS

post-column reaction; rat; brain; SPE

REFERENCE

Ohno,M.; Kai,M.; Ohkura,Y. High-performance liquid chromatographic determination of substance P-like arginine-containing peptide in rat brain by on-line post-column fluorescence derivatization with benzoin, *J.Chromatogr.*, **1989**, *490*, 301–310.

SAMPLE

Matrix: tissue

Sample preparation: Tissue homogenate + MeCN, vortex for 10 s, centrifuge at 10000 g for 15 min. Remove the supernatant and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 100 μL mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4 10 μm Lichrosorb RP-18

Mobile phase: Gradient. MeCN:0.6% aqueous ethanolamine, pH 3.0 14:86 for 5 min, to 30:70 over 8 min, maintain at 30:70 for 7 min, re-equilibrate at initial conditions for 5 min.

Flow rate: 1

Detector: UV 254

CHROMATOGRAM
Retention time: 10

OTHER SUBSTANCES
Extracted: degradation products

KEY WORDS
rabbit; rectal mucosa; vaginal mucosa; nasal mucosa

REFERENCE
Han,K.; Park,J.S.; Chung,Y.B.; Lee,M.J.; Moon,D.C.; Robinson,J.R. Identification of enzymatic degradation products of luteinizing hormone releasing hormone (LHRH)/[D-Ala6] LHRH in rabbit mucosal homogenates, *Pharm.Res.*, **1995**, *12*, 1539–1544.

Gonadotropin

Molecular weight: ca. 39500
CAS Registry No.: 9002-71-3
Merck Index: 2273

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 4 10 μm μBondapak alkylphenyl
Mobile phase: Gradient. A was 100 mM pH 7.0 ammonium acetate. B was MeCN:water 60:40 containing 15 mM trifluoroacetic acid, pH 2.0. A:B from 100:0 to 0:100 over 90 min.
Flow rate: 1.2
Detector: UV 278

CHROMATOGRAM
Retention time: 30 (α1), 32 (α2), 33 (β1), 34 (β2) (subunits)

REFERENCE
Grego,B.; Hearn,M.T.W. High-performance liquid chromatography of amino acids, peptides and proteins. LXIII. Reversed-phase high-performance liquid chromatographic characterisation of several polypeptide and protein hormones, *J.Chromatogr.*, **1984**, *336*, 25–40.

SAMPLE
Matrix: solutions

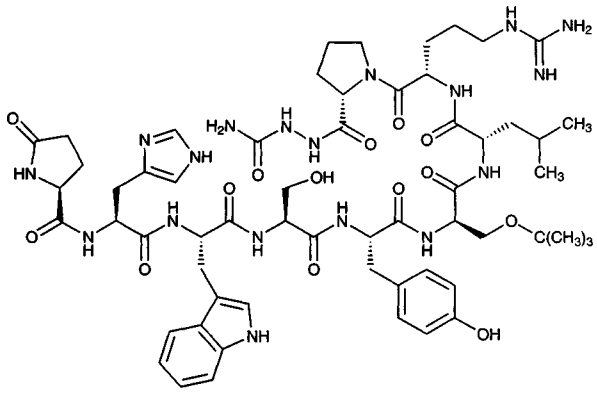
HPLC VARIABLES
Column: 250 × 4.6 Vydac C4 300 A no. 214TP54
Mobile phase: Gradient. A was 0.1% trifluoroacetic acid in water. B was 0.1% trifluoroacetic acid in MeCN. A:B from 100:0 to 40:60 over 90 min.
Flow rate: 1
Detector: UV 220

CHROMATOGRAM
Retention time: 50 (α-1), 52 (α-2), 50 (β-1), 54 (β-2) (subunits)

REFERENCE
Pollak,S.; Halpine,S.; Chait,B.T.; Birken,S. High resolution high performance liquid chromatography fingerprinting of purified human chorionic gonadotropin demonstrates that oxidation is a cause of hormone heterogeneity, *Endocrinology*, **1990**, *126*, 199–208.

Goserelin

Molecular formula: $C_{59}H_{84}N_{18}O_{14}$
Molecular weight: 1269.43
CAS Registry No.: 65807-02-5
Merck Index: 4547



SAMPLE
Matrix: solutions

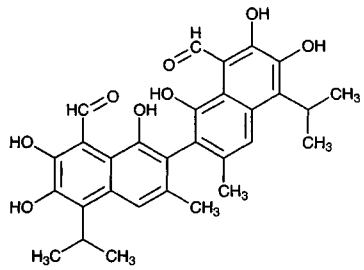
HPLC VARIABLES
Column: 125 × 4 5 μm Lichrosphere 100 RP-18
Mobile phase: MeCN:0.1% trifluoroacetic acid 22:78
Flow rate: 1
Detector: UV 214

OTHER SUBSTANCES
Simultaneous: degradation products
Also analyzed: triptorelin

REFERENCE
Hoitink,M.A.; Beijnen,J.H.; Boschma,M.U.S.; Bult,A.; Hop,E.; Nijholt,J.; Versluis,C.; Wiese,G.; Underberg,W.J.M. Identification of the degradation products of gonadorelin and three analogues in aqueous solution, *Anal.Chem.*, **1997**, *69*, 4972–4978.

Gossypol

Molecular formula: $C_{30}H_{30}O_8$
Molecular weight: 518.56
CAS Registry No.: 303-45-7
Merck Index: 4549



SAMPLE
Matrix: blood
Sample preparation: 400 μL serum + 500 μL saturated disodium EDTA, mix, let stand for 10 min, adjust to pH 8.0 with concentrated NaOH solution using a micro-electrode, add 50 μL 1 g/mL L-phenylalanine methyl ester in MeCN, let stand for 10 min, adjust pH to 7.00 with concentrated sulfuric acid, add 2 mL ether, vortex, centrifuge, repeat extraction. Combine the organic layers and evaporate them to dryness under reduced pressure, reconstitute the residue in 500 μL MeCN, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.5 5 μm Hypersil ODS

Mobile phase: MeCN:THF:buffer 76:2:22 (Buffer was 10 mM KH_2PO_4 adjusted to pH 2.35 with phosphoric acid.)
Flow rate: 2.5
Injection volume: 50
Detector: UV 250

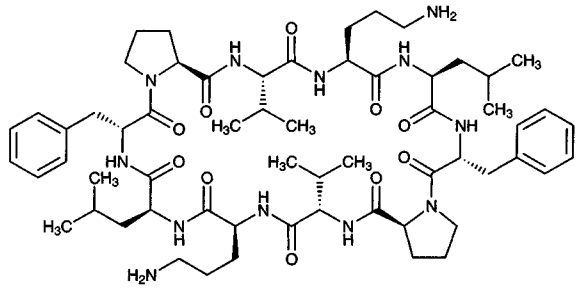
CHROMATOGRAM
Retention time: 11 (-), 17 (+)
Limit of detection: 30 ng/mL

KEY WORDS
serum; derivatization; chiral

REFERENCE
Matlin,S.A.; Belenguer,A.; Vince,P.M.; Stein,R. Analysis of gossypol enantiomers in human serum, *J.Liq.Chromatogr.*, **1990**, *13*, 2261–2268.

Gramicidin

Molecular formula: $C_{60}H_{92}N_{12}O_{10}$
Molecular weight: 1141.47
CAS Registry No.: 1405-97-6, 113-73-5 (gramicidin S)
Merck Index: 4552



SAMPLE
Matrix: saliva
Sample preparation: Mix saliva with an equal volume of EtOH, centrifuge.

HPLC VARIABLES
Column: 200 × 3 5 μm Nucleosil C8
Mobile phase: MeCN:water:phosphoric acid 47.5:52.5:0.01
Column temperature: 70
Flow rate: 2.5
Injection volume: 20
Detector: UV 220

CHROMATOGRAM
Retention time: 13 (valin-gramicidin A)
Limit of detection: 1 μg/mL

KEY WORDS
pharmacokinetics

REFERENCE
Kreuzig,F.; Nahler,G. Salivary levels of gramicidin after use of a tyrothricin lozenge and a tyrothricin gargle/mouth-wash, *Int.J.Clin.Pharmacol.Res.*, **1983**, *3*, 65–70.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 2 μL aliquot.

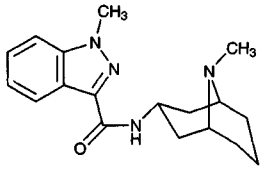
HPLC VARIABLES
Column: 300 × 7.8 Ultrastyragel 1000 Å (Waters)

Mobile phase: THF
Flow rate: 1
Injection volume: 2
Detector: F ex 297 em 330

CHROMATOGRAM
Retention time: 7.9 (dimers), 8.4 (monomer)

REFERENCE
Bañó,M.C.; Braco,L.; Abad,C. New high-performance liquid chromatography-based methodology for monitoring the conformational transitions of self-associating hydrophobic peptides, incorporated into liposomes, *J.Chromatogr.*, **1988**, *458*, 105–116.

Granisetron



Molecular formula: $C_{18}H_{24}N_4O$
Molecular weight: 312.41
CAS Registry No.: 109889-09-0, 107007-99-8 (HCl)
Merck Index: 4557
Lednicer No.: 5 118

SAMPLE
Matrix: blood
Sample preparation: Filter (Amicon Centricon-10 microconcentrator, 10000 daltons molecular weight cutoff) 150 μL plasma while centrifuging at 5° at 5000 g for 100 min, inject a 10 μL aliquot of the ultrafiltrate.

HPLC VARIABLES
Column: 100 × 4.6 5 μm Spheri-5 silica
Mobile phase: MeCN:25 mM pH 4.2 sodium acetate 40:60
Flow rate: 1
Injection volume: 10
Detector: UV 305

CHROMATOGRAM
Retention time: 3.4
Limit of detection: 19 ng/mL

KEY WORDS
plasma; guinea pig; ultrafiltrate

REFERENCE
Capacio,B.R.; Byers,C.E.; Jackson,T.K.; Matthews,R.L. An HPLC method for the determination of granisetron in guinea pig plasma, *J.Anal.Toxicol.*, **1993**, *17*, 151–155.

SAMPLE
Matrix: blood
Sample preparation: Condition a 100 mg Bond Elut C2 SPE cartridge with two 1 mL portions of MeOH, 1 mL water, and two 1 mL portions of buffer. 1 mL Plasma + 100 μL 40 ng/mL IS in water + 500 μL buffer, add to the SPE cartridge, wash with 1 mL MeCN:water 40:60, remove all wash solvent, elute with 800 μL MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 50°. Add 30 μL 10% trimethylsilyldiazomethane in hexane and 30 μL 2% N,N-diisopropylethylamine in MeOH to the residue, heat at 50° for 20 min, cool to room temperature, evaporate to dryness under a stream of nitrogen at 50°, reconstitute in 300 μL MeOH:water 10:90, centrifuge at 1700 g for 5 min, inject a 150 μL aliquot. (Phosphate buffer was 4.33 g Na_2HPO_4 and 3.04 g $NaH_2PO_4.2H_2O$ in 50 mL water.)

HPLC VARIABLES
Guard column: 15 × 3.2 NewGuard RP-18
Column: 250 × 4.6 5 μm Develosil ODS-5 (Nomura Chemical)
Mobile phase: MeOH:buffer 30:70 (Buffer was 15.4 g ammonium acetate and 20 mL tetra-n-butylammonium hydroxide in 1.7 L water, adjust pH to 4.70 with glacial acetic acid, make up to 2 L with water.)
Column temperature: 45
Flow rate: 1
Injection volume: 150
Detector: F ex 310 em 420

CHROMATOGRAM
Retention time: 9.5
Internal standard: 2-methyl-M-(endo-9-methyl-9-azabicyclo[3.3.1]non-3-yl)-2H-indazole-3-carboxamide hydrochloride (6)
Limit of detection: 42 pg/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; SPE; derivatization

REFERENCE
Kudoh,S.; Sato,T.; Okada,H.; Kumakura,H.; Nakamura,H. Simultaneous determination of granisetron and 7-hydroxygranisetron in human plasma by high-performance liquid chromatography with fluorescence detection, *J.Chromatogr.B*, **1994**, *660*, 205–210.

SAMPLE
Matrix: blood
Sample preparation: Condition a C2 SPE cartridge (Analytichem) with 1 mL MeOH, 1 mL water, and 1 mL 1 M pH 7.0 phosphate buffer. 1 mL Plasma + 50 μL water + 50 μL 100 ng/mL IS in water + 500 μL 1 M pH 7.0 phosphate buffer, add immediately to SPE cartridge, wash with 1 mL water, wash with 1 mL MeCN:water 40:60, remove wash solvent completely, elute with 1 mL MeOH, elute with 1 mL MeOH:trifluoroacetic acid 99:1. Combine the eluates and evaporate them to dryness under a stream of nitrogen at 40°, reconstitute the residue in 200 μL MeOH:water 10:90, vortex for 30 s, centrifuge at 2000 g for 5 min, transfer to a fresh vial, centrifuge at 2000 g for 5 min, inject a 10-65 μL aliquot.

HPLC VARIABLES
Guard column: 30 × 2.1 C8 (ABI Instruments)
Column: 150 × 2.1 Zorbax Rx C8
Mobile phase: MeCN:buffer 19:81 (Buffer was 0.95 g sodium hexanesulfonate in 405 mL 100 mM pH 4.7 acetate buffer.)
Column temperature: 30
Flow rate: 0.3
Injection volume: 10-65
Detector: E, ESA, E1 0.15 V, E2 0.35 V (measuring electrode), guard cell 0.4 V (before injector) followed by F ex 305 em 360

CHROMATOGRAM
Retention time: 17 (F)
Internal standard: 8-methyl-8-azabicyclo[3.2.1]oct-3-yl 1-methyl-1H-indazole-3-carboxylate (BRL 43704) (21) (F)
Limit of quantitation: 0.1 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; SPE; fluorescence detection for granisetron and some metabolites; electrochemical detection for other metabolites

REFERENCE

Boppana,V.K. Simultaneous determination of granisetron and its 7-hydroxy metabolite in human plasma by reversed-phase high-performance liquid chromatography utilizing fluorescence and electrochemical detection, *J.Chromatogr.A*, **1995**, *692*, 195–202.

SAMPLE

Matrix: blood

Sample preparation: 500 μL Plasma + 7 μg IS, vortex for 10 s, add 1.5 mL toluene, add 250 μL buffer, shake for 20 min, centrifuge at 3000 g for 10 min. Remove 1 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 37°, reconstitute the residue in 40 μL mobile phase, vortex for 10 s, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 10 μm Spherisorb CN

Mobile phase: MeCN:buffer 15:85 (Buffer was 100 mM NaH_2PO_4 adjusted to pH 4.5 with orthophosphoric acid.)

Flow rate: 2

Injection volume: 20

Detector: F ex 305 em 365

CHROMATOGRAM

Retention time: 6.64

Internal standard: N-(1-naphthyl)ethylenediamine dihydrochloride (3.60)

Limit of detection: 0.1 ng/mL

Limit of quantitation: 0.3 ng/mL

OTHER SUBSTANCES

Noninterfering: metabolites, anthracyclines, cimetidine, cisplatin, dexamethasone, etoposide, fluorouracil, methotrexate, methylprednisolone, ondansetron

KEY WORDS

plasma

REFERENCE

Pinguet,F.; Bressolle,F.; Martel,P.; Salabert,D.; Astre,C. High-performance liquid chromatographic determination of granisetron in human plasma, *J.Chromatogr.B*, **1996**, *675*, 99–105.

SAMPLE

Matrix: blood, pleural effusion, urine

Sample preparation: Mix 500 μL serum, pleural effusion, or urine with 100 μL IS solution and 400 μL pH 9.0 ammonium acetate. Add to an Extrelut-1 SPE cartridge. After 15 min, elute with 3 mL and 4 mL portions of dichloromethane. Evaporate the eluate to dryness under a stream of nitrogen at 40°, reconstitute the residue with 100 μL mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 10 μm LiChroCART LiChrospher 100 CN

Mobile phase: MeCN:100 mM pH 3.5 acetate buffer 30:70

Column temperature: 40

Flow rate: 1

Injection volume: 20

Detector: F ex 290 em 365

CHROMATOGRAM

Retention time: 11.3

Internal standard: BRL 43693A (Smith Kline Beecham) (9.5)

Limit of detection: 250 pg/mL

Limit of quantitation: 2 ng/mL

OTHER SUBSTANCES

Extracted: etoposide

Simultaneous: domperidone, metoclopramide, ondansetron

Noninterfering: cisplatin, carboplatin, dexamethasone

KEY WORDS
serum; SPE; pharmacokinetics

REFERENCE
Wada,I.; Satoh,M.; Takeda,T.; Nakabayashi,T.; Honma,T.; Saitoh,H.; Takada,M.; Hirano,K. A rapid assay of granisetron in biological fluids from cancer patients, *Biol.Pharm.Bull.*, **1998**, *21*, 535–537.

SAMPLE
Matrix: blood, urine
Sample preparation: 1 mL Plasma or urine + 100 μL IS in water + 500 μL 100 mM pH 12 phosphate buffer + 3 mL toluene, shake mechanically for 30 min, centrifuge. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 60°, reconstitute the residue in 100 μL mobile phase, inject an 80 μL aliquot.

HPLC VARIABLES
Guard column: 50 mm long unspecified
Column: 250 X 4.5 10 μM Apex CN
Mobile phase: MeOH:buffer 97:3 (Buffer was 50 mM sodium acetate containing 0.25% triethylamine adjusted to pH 6.0.)
Flow rate: 1
Injection volume: 80
Detector: F ex 305 em 360

CHROMATOGRAM
Retention time: 13.5
Internal standard: endo-1-methyl-O-(9-methyl-9-azabicyclo(3,3,1)non-3-yl)-1H-indazole-3-carboxylate (BRL 43704) (19.2)
Limit of detection: 0.1 ng/mL

KEY WORDS
plasma; human; rat; dog

REFERENCE
Clarkson,A.; Coates,P.E.; Zussman,B.D. A specific h.p.l.c. method for the determination of BRL 43694 in plasma and urine, *Br.J.Clin.Pharmacol.*, **1988**, *25*, 136P.

SAMPLE
Matrix: formulations
Sample preparation: Dilute 1-mg granisetron hydrochloride injection with 0.9% NaCl or 5% dextrose to a granisetron concentration of 100 μg/mL, inject a 20 μL aliquot of the solution.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Spherisorb Cyano
Mobile phase: MeCN:100 mM NaH_2PO_4 15:85
Flow rate: 2
Injection volume: 20
Detector: UV 302

CHROMATOGRAM
Retention time: 9.1

OTHER SUBSTANCES
Simultaneous: degradation products

KEY WORDS
injections; stability-indicating

REFERENCE
Quercia,R.A.; Zhang,J.; Fan,C.; Chow,M.S.S. Stability of granisetron hydrochloride in polypropylene syringes, *Am.J.Health-Syst.Pharm.*, **1996**, *53*, 2744–2746.

SAMPLE
Matrix: formulations
Sample preparation: Filter (0.2 μm nylon), inject a 50 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Accubond CN (J & W)
Mobile phase: MeCN:buffer 60:40 (Buffer was 20 mM KH_2PO_4 containing 5 mM octanesulfonic acid, adjusted to pH 6.0 with 1 M NaOH.)
Flow rate: 1.5
Injection volume: 50
Detector: UV 305

CHROMATOGRAM
Retention time: 7.5

KEY WORDS
injections; saline; 5% dextrose; stability-indicating

REFERENCE
Chung,K.C.; Chin,M.A.; Gill,M.A. Stability of granisetron hydrochloride in a disposable elastomeric infusion device, *Am.J.Health-Syst.Pharm.*, **1995**, *52*, 1541–1543.

SAMPLE
Matrix: formulations
Sample preparation: Inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Spherisorb CN
Mobile phase: MeCN:100 mM pH 4.5 NaH_2PO_4 15:85
Injection volume: 20
Detector: UV 300

CHROMATOGRAM
Retention time: 6.8
Internal standard: propylparaben (3.3)

OTHER SUBSTANCES
Simultaneous: dexamethasone, methylprednisolone

KEY WORDS
injections; 5% dextrose; saline; water

REFERENCE
Pinguet,F.; Rouanet,P.; Martel,P.; Fabbro,M.; Salabert,D.; Astre,C. Compatibility and stability of granisetron, dexamethasone, and methylprednisolone in injectable solutions, *J.Pharm.Sci.*, **1995**, *84*, 267–268.

SAMPLE
Matrix: formulations
Sample preparation: Dilute with mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 μm cyano
Mobile phase: MeCN:100 mM NaH_2PO_4 20:80 adjusted to pH 4.2 with phosphoric acid
Flow rate: 2
Injection volume: 20

Detector: UV 300

CHROMATOGRAM
Retention time: 5.60

OTHER SUBSTANCES
Simultaneous: dexamethasone (UV 228)

KEY WORDS
stability-indicating; injections; saline; 5% dextrose; apple juice; orange juice; soft drinks

REFERENCE
Mayron,D.; Gennaro,A.R. Stability and compatibility of granisetron hydrochloride in i.v. solutions and oral liquids and during simulated Y-site injection with selected drugs, *Am.J.Health-Syst.Pharm.*, **1996**, *53*, 294–304.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 3.9 5 μm Symmetry C8 (Waters)
Mobile phase: MeCN:20 mM sodium dihydrogen phosphate 20:80
Flow rate: 1
Injection volume: 20
Detector: UV 307

CHROMATOGRAM
Retention time: 4.5

OTHER SUBSTANCES
Simultaneous: doxorubicin (11.2)

REFERENCE
Zhang,H.; Ye,L.; Stewart,J.T. HPLC determinations of doxorubicin with selected medications in 0.9% sodium chloride injection USP, *J.Liq.Chromatogr.Rel.Technol.*, **1998**, *21*, 2375–2385.

SAMPLE
Matrix: solutions
Sample preparation: Inject an aliquot of an aqueous solution.

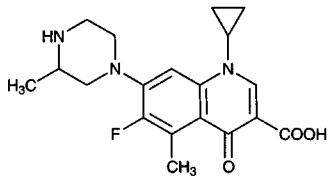
HPLC VARIABLES
Column: 250 × 4 Nucleosil C18
Mobile phase: MeOH:THF:buffer 30:5:65 (Buffer was 100 mM triethylamine adjusted to pH 3.0 with nitric acid.)
Flow rate: 0.8
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 6.37

OTHER SUBSTANCES
Simultaneous: ondansetron, tropisetron

REFERENCE
Barbato,F.; Immacolata La Rotonda,M.; Quaglia,F. Retention behaviour of anti-emetic serotonin antagonists in reversed phase high performance liquid chromatography, *Farmaco*, **1995**, *50*, 875–880.

Grepafloxacin



Molecular formula: $C_{19}H_{22}FN_3O_3$
Molecular weight: 359.40
CAS Registry No.: 119914-60-2, 146863-02-7 (±), 161967-81-3 ((±) HCl)
Merck Index: 4567

SAMPLE
Matrix: blood
Sample preparation: Extract 200 µL plasma with dichloromethane:n-butanol 95:5 and evaporate to dryness. Treat the organic layer residue with Mosher's acid chloride (R-(-)- or S-(+)-α-methoxy-α -trifluoromethylphenylacetic acid chloride) and triethanolamine in dichloromethane for 1 hr. Reconstitute, inject an aliquot.

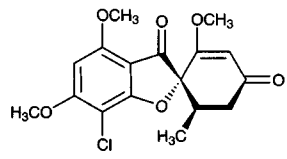
HPLC VARIABLES
Column: 150 × 3.2 5µm ODS
Mobile phase: MeCN:0.2% phosphoric acid 70:30
Column temperature: 35
Detector: F ex 290 em 470

CHROMATOGRAM
Retention time: 12.7 (S-(-)), 13.5 (R-(+))
Internal standard: ciprofloxacin (7.4)
Limit of quantitation: 25 ng/mL

KEY WORDS
derivatization; chiral; plasma

REFERENCE
Tata,P.N.V.; Bramer,S.L. Enantiomeric assay of grepafloxacin in plasma (Abstract 4162), *Pharm.Res.*, **1997**, *14*, S684.

Griseofulvin



Molecular formula: $C_{17}H_{17}ClO_6$
Molecular weight: 352.8
CAS Registry No.: 126-07-8
Merck Index: 4571
Lednicer No.: 1 314

SAMPLE
Matrix: blood
Sample preparation: 100 µL Plasma + 100 µL p-phenylphenol in MeCN, vortex, centrifuge, inject a 20 µL aliquot of the supernatant.

HPLC VARIABLES
Column: 150 mm long 5 µm Novapack
Mobile phase: MeCN:0.1 M acetic acid, pH 3.5 45:55
Flow rate: 1
Injection volume: 20
Detector: UV 290

CHROMATOGRAM
Retention time: 3.0

Internal standard: p-phenylphenol (4.5)
Limit of detection: 50 ng/mL

KEY WORDS
rat; plasma; pharmacokinetics

REFERENCE
Vudathala,G.K.; Rogers,J.A. Oral bioavailability of griseofulvin from aged griseofulvin: lipid coprecipitates: in vivo studies in rats, *J.Pharm.Sci.*, **1992**, *81*, 1166–1169.

SAMPLE
Matrix: blood, CSF, gastric contents, urine
Sample preparation: 200 μL Serum, urine, CSF, or gastric fluid + 300 μL reagent. Flush column A to waste with 500 μL 500 mM ammonium sulfate, inject sample onto column A, flush column A to waste with 500 μL 500 mM ammonium sulfate, backflush the contents of column A onto column B with mobile phase, monitor the effluent from column B. (Reagent was 8.05 M guanidine HCl and 1.02 M ammonium sulfate in water.)

HPLC VARIABLES
Column: A 40 μm preparative grade C18 (Analytichem); B 75 × 2.1 pellicular C18 (Whatman) + 250 × 4.6 5 μm C8 end-capped (Whatman)
Mobile phase: Gradient. A was 50 mM pH 4.5 KH_2PO_4. B was MeCN:isopropanol 80:20. A:B 90:10 for 1 min, to 30:70 over 20 min.
Column temperature: 50
Flow rate: 1.5
Detector: UV 220

CHROMATOGRAM
Retention time: 13.51
Internal standard: heptanophenone (19)

OTHER SUBSTANCES
Extracted: acetaminophen, allobarbital, azinphos, barbital, brallobarbitone, bromazepam, butethal, caffeine, carbamazepine, carbaryl, cephaloridine, chloramphenicol, chlordiazepoxide, chlorothiazide, chlorvinphos, clothiapine, cocaine, coomassie blue, desipramine, diazepam, diphenhydramine, dipipanone, ethylbromphos, flufenamic acid, formothion, indomethacin, lidocaine, lorazepam, malathion, medazepam, midazolam, oxazepam, paraoxon, penicillin G, pentobarbital, prazepam, propoxyphene, prothiophos, quinine, salicylic acid, secobarbital, strychnine, sulfamethoxazole, theophylline, thiopental, thioridazine, trimethoprim

KEY WORDS
serum; column-switching

REFERENCE
Kruger,P.B.; Albrecht,C.F.De V.; Jaarsveld,P.P. Use of guanidine hydrochloride and ammonium sulfate in comprehensive in-line sorption enrichment of xenobiotics in biological fluids by high-performance liquid chromatography, *J.Chromatogr.*, **1993**, *612*, 191–198.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 292.6

CHROMATOGRAM
Retention time: 18.392

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: solutions
Sample preparation: Mix an excess of griseofulvin with 8 mL water or with 8 mL 6-7 mM sodium dodecyl sulfate. Shake in a 25° water bath for 48 h, filter through a 0.45 μm filter. Discard the first a few mL, inject a 150 μL aliquot of the rest.

HPLC VARIABLES
Column: 140 × 4.6 5μm cyanopropyl methyl silane bonded silica (Supelco Inc., PA)
Mobile phase: MeOH:water 50:50
Flow rate: 1
Injection volume: 150
Detector: UV 295

REFERENCE
Rao,V.M.; Lin,M.; Larive,C.K.; Southard,M.Z. A mechanistic study of griseofulvin dissolution into surfactant solutions under laminar flow conditions, *J.Pharm.Sci.*, **1997**, *86*, 1132–1137.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 100 × 4.6 5 μm Spherisorb 5 ODS
Mobile phase: MeCN:10 mM phosphoric acid 34:66, pH 2.82
Column temperature: 40
Flow rate: 1
Injection volume: 50
Detector: UV 248

CHROMATOGRAM
Retention time: 12.13
Internal standard: griseofulvin

OTHER SUBSTANCES
Simultaneous: methoxsalen

KEY WORDS
SPE; griseofulvin is IS

REFERENCE
Kucová,D.; Marysková,D.; Davídková,P.; Gasparic,J. High-performance liquid chromatographic determination of methoxsalen in plasma after liquid-solid extraction, *J.Chromatogr.*, **1993**, *614*, 340–344.

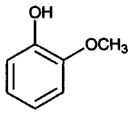
SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in MeOH, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 μm RP 18
Mobile phase: MeOH:water 60:40
Detector: UV 245 and 315

CHROMATOGRAM
Limit of quantitation: 500 ng/mL

REFERENCE
De Smidt,J.H.; Grit,M.; Crommelin,D.J.A. Dissolution kinetics of griseofulvin in mixed micellar solutions, *J.Pharm.Sci.*, **1994**, *83*, 1209–1212.

Guaiacol



Molecular formula: $C_7H_8O_2$
Molecular weight: 124.14
CAS Registry No.: 90-05-1
Merck Index: 4575

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 13.787

KEY WORDS
whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE

Matrix: solutions

HPLC VARIABLES

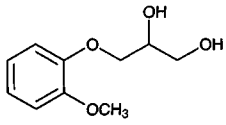
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE
Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Guaifenesin



Molecular formula: $C_{10}H_{14}O_4$
Molecular weight: 198.22
CAS Registry No.: 93-14-1
Merck Index: 4582
Lednicer No.: 1 118

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 100 μL 2.5 mg/mL O-desmethylnaproxen in MeOH + 400 μL acetone, homogenize for 10 min, centrifuge at 1000 g for 15 min. Remove supernatant and evaporate it to dryness under a stream of air at 35°. Take up residue in 500 μL mobile phase, inject 10 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm LiChrosorb RP-18
Mobile phase: MeOH:10 mM pH 6.5 citrate buffer 10:90
Column temperature: 35
Flow rate: 2
Injection volume: 10
Detector: F ex 230 em 306

CHROMATOGRAM
Retention time: 37
Internal standard: O-desmethylnaproxen (26)

OTHER SUBSTANCES
Simultaneous: metabolites

KEY WORDS
plasma; horse

REFERENCE
Ketelaars,H.C.; Peters,J.G.; Anzion,R.B.; Van Ginneken,C.A. Isolation, partial identification and quantitative determination of four guaiphenesin glucuronides in plasma and urine of the horse by high-performance liquid chromatography, *J.Chromatogr.*, **1984**, *288*, 423–429.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 11.435

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: bulk
Sample preparation: Dissolve in mobile phase, inject a 20 μL aliquot.

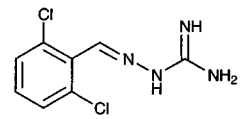
HPLC VARIABLES
Column: 250 × 4.6 10 μm Chiralcel OD
Mobile phase: EtOH:heptane 20:80
Flow rate: 1
Injection volume: 20
Detector: UV 365

CHROMATOGRAM
Retention time: k' 0.57 ((R)-(-)), k'1.72 ((S)-(+))

KEY WORDS
chiral

REFERENCE
Francotte,E.R.; Richert,P. Applications of simulated moving-bed chromatography to the separation of the enantiomers of chiral drugs, *J.Chromatogr.A*, **1997**, *769*, 101–107.

Guanabenz



Molecular formula: $C_8H_8Cl_2N_4$
Molecular weight: 231.08
CAS Registry No.: 5051-62-7, 23256-50-0 (acetate)
Merck Index: 4585
Lednicer No.: 2 123

SAMPLE
Matrix: formulations
Sample preparation: Finely powder tablets, weigh out amount equivalent to 30 mg guanabenz acetate, add 190 mL MeCN, sonicate for a few minutes, make up to 200 mL with MeCN, filter. Remove a 14-59 mL aliquot and add it to 9 mL 1 mg/mL carbamazepine in MeCN, make up to 100 mL with MeCN, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 μm octadecylsilane (Perkin-Elmer)
Mobile phase: MeCN:buffer 35:65 (Buffer was 4 mM KH_2PO_4 adjusted to pH 3.25 with phosphoric acid.)
Flow rate: 2.5
Injection volume: 20
Detector: UV 265

CHROMATOGRAM
Retention time: 2
Internal standard: carbamazepine (3)

OTHER SUBSTANCES
Simultaneous: mefruside, impurities

KEY WORDS
tablets

REFERENCE
Vio,L.; Mamolo,M.G.; Furlan,G. Quantitative high pressure liquid chromatographic determination of guanabenz and mephruside in pharmaceutical formulations, *Farmaco.[Prat].*, **1988**, *43*, 27–36.

SAMPLE
Matrix: formulations
Sample preparation: Dilute a 500 μL aliquot of syrup with water to give a clonidine concentration of 5 μg/mL, filter (0.22 μm), inject a 15 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Zorbax TMS trimethylsilyl
Mobile phase: MeOH:buffer 65:35 (Buffer was 2.2 mM KH_2PO_4 and 16 mM Na_2HPO_4, pH 7.9.)
Flow rate: 1
Injection volume: 15
Detector: UV 254

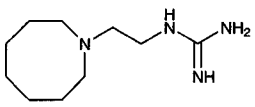
CHROMATOGRAM
Retention time: 7
Internal standard: guanabenz

OTHER SUBSTANCES
Simultaneous: clonidine

KEY WORDS
syrup; guanabenz is IS

REFERENCE
Levinson,M.L.; Johnson,C.E. Stability of an extemporaneously compounded clonidine hydrochloride oral liquid, *Am.J.Hosp.Pharm.*, **1992**, *49*, 122–125.

Guanethidine



Molecular formula: $C_{10}H_{22}N_4$
Molecular weight: 198.31
CAS Registry No.: 55-65-2, 645-43-2 (monosulfate), 60-02-6 (sulfate)
Merck Index: 4589
Lednicer No.: 1 282

SAMPLE

Matrix: blood
Sample preparation: Inject 200 μL serum onto column A and elute to waste with mobile phase A, after 2 min elute the contents of column A onto column B with mobile phase B, after another 3 min remove column A from the circuit, elute column B with mobile phase B, monitor the effluent from column B. Flush column A with mobile phase C for 10 min, re-equilibrate with mobile phase A for 7 min.

HPLC VARIABLES

Column: A 35 × 4.6 10 μm TSK precolumn BSA-ODS (Tosoh); B 150 × 4.6 5 μm TSKgel ODS-80TM (Tosoh)
Mobile phase: A 50 mM NaH_2PO_4 adjusted to pH 3.0 with 50 mM phosphoric acid; B MeCN:buffer 30:70, containing 7 g/L sodium 1-octanesulfonate (Buffer was 50 mM NaH_2PO_4 adjusted to pH 3.0 with 50 mM phosphoric acid.); C MeCN:water 50:50.
Flow rate: 1
Injection volume: 200
Detector: F ex 39 em 500 following post-column reaction. The effluent from column B mixed with 1 M NaOH pumped at 0.3 mL/min and with 6 g/L ninhydrin in water pumped at 0.3 mL/min and the mixture flowed through a 10 m × 0.5 mm ID PTFE coil at 56° to the detector.

CHROMATOGRAM

Retention time: 16
Limit of detection: 1 ng/mL
Limit of quantitation: 3.1 ng/mL

KEY WORDS

serum; post-column reaction; column-switching; rat; human; pharmacokinetics

REFERENCE

Inamoto,Y.; Inamoto,S.; Hanai,T.; Takahashi,Y.; Kadowaki,K.; Kinoshita,T. Development of automated highly sensitive analytical system for guanethidine sulfate in serum, *J.Liq.Chromatogr.Rel.Technol.*, **1997**, *20*, 2099–2108.

SAMPLE

Matrix: formulations
Sample preparation: Grind tablets, add 3-20 mL MeCN:water 15:85, sonicate for 10 min, filter, make up to 100 mL with MeCN:water 15:85. Remove a 500 μL aliquot and add it to 300 μL 250 μg/mL procaine hydrochloride in water, make up to 10 mL with mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm ASI chromosphere 3869 octadecylsilane (Analytical Sciences, Inc.)
Mobile phase: MeCN:50 mM NaH_2PO_4 30:70 containing sodium pentanesulfonate, pH adjusted to 2.5 with concentrated phosphoric acid
Flow rate: 1
Injection volume: 50
Detector: E, Metrohm model E-611, Bioanalytical Systems Kel F cell, glassy carbon electrode + 1300 mV, auxiliary platinum electrode, Ag/AgCl reference electrode

CHROMATOGRAM

Retention time: 3.5

Internal standard: procaine hydrochloride (5.7)
Limit of quantitation: 500 ng/mL

OTHER SUBSTANCES
Simultaneous: hydrochlorothiazide

KEY WORDS
tablets; not stability-indicating

REFERENCE
Stewart,J.T.; Clark,S.S. Liquid chromatographic determination of guanethidine salts and hydrochlorothiazide using electrochemical detection and ion-pair techniques, *J.Pharm.Sci.*, **1986**, *75*, 413–415.

SAMPLE
Matrix: solutions

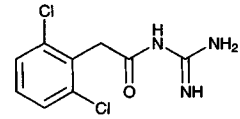
HPLC VARIABLES
Column: μBondapak CN
Mobile phase: MeCN:0.1% pH 7.36 ammonium acetate 50:50
Flow rate: 2
Detector: UV 206

CHROMATOGRAM
Retention time: 2
Limit of detection: 20 ng

KEY WORDS
rabbit; buffer

REFERENCE
Tang-Liu,D.D.-S.; Richman,J.B.; Weinkam,R.J.; Takruri,H. Effects of four penetration enhancers on corneal permeability of drugs in vitro, *J.Pharm.Sci.*, **1994**, *83*, 85–90.

Guanfacine



Molecular formula: $C_9H_9Cl_2N_3O$
Molecular weight: 246.10
CAS Registry No.: 29110-47-2, 29110-48-3 (HCl)
Merck Index: 4590
Lednicer No.: 3 40

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

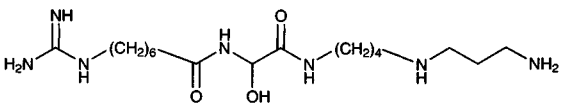
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 11.387

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Gusperimus



Molecular formula: $C_{17}H_{37}N_7O_3$
Molecular weight: 387.53
CAS Registry No.: 104317-84-2, 84937-45-1 ((-)-form tri HCl), 85468-01-5 (tri HCl)
Merck Index: 4610

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 10 μg IS, mix well, make up to 10 mL with water, add to a 50 × 6 column of CM-Sephadex C-25, wash with 10 mL 300 mM NaCl, elute with 10 mL 400 mM NaCl, add the eluate to a Sep-Pak C18 SPE cartridge, wash with 5 mL water, elute with 10 mL MeOH. Evaporate the eluate to dryness under reduced pressure, reconstitute the residue in 200 μL mobile phase, vortex, inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: Guard Pak C18 (Waters)
Column: 150 × 4.6 Cosmosil 5C18-P (Nacalai Tesque)
Mobile phase: MeCN:buffer 9:91 (Buffer was 10 mM pH 3 phosphate buffer containing 5 mM sodium pentanesulfonate.)
Flow rate: 1
Injection volume: 50
Detector: UV 205

CHROMATOGRAM
Retention time: 8.6
Internal standard: 1-amino-20-guanidino-11-hydroxy-4,9,12-triazaeicosane-10,13-dione (Heat 33.7 mmole 8-guanidinooctanamide, 30.7 mmole glyoxylspermidine hydrochloride, 33.7 mmole glutaric acid, and 2 g water at 60° for 8 h, dilute with water, chromatograph on CM-Sephadex C-25(Na^+) using gradient elution with water and 1 M NaCl. Evaporate the eluate fraction containing the product to dryness, extract the residue with MeOH, chromatograph the extract on Sephadex LH-20 with MeOH, evaporate the eluate to dryness to get the compound.) (18.6)
Limit of quantitation: 50 ng/mL

KEY WORDS
dog; plasma; SPE; pharmacokinetics

REFERENCE
Nakanuma,R.; Watanabe,K.; Yamashita,K.; Mizuguchi,S.; Hashimoto,Y.; Nakamura,T.; Umezawa,H. High-performance liquid chromatographic determination of deoxyspergualin in dog plasma with ultraviolet detection, *J.Chromatogr.*, **1990**, *527*, 208–213.

SAMPLE
Matrix: blood
Sample preparation: Filter (Amicon MPS-1 with 14 mm YM20 membrane) 1 mL plasma while centrifuging at 800 g for 1 h. Remove a 400 μL aliquot of the ultrafiltrate and add it to 50 μL 500 mM pH 6.8 phosphate buffer, add 50 μL 10 mM NaCN in water, add 100 μL 2 mM naphthalene-2,3-dicarboxaldehyde in MeCN, let stand at room temperature for 15 min, add 50 μL 500 mM pH 3.0 sodium acetate buffer, inject an aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm ODS Hypersil
Mobile phase: MeCN:100 mM KH_2PO_4:phosphoric acid 52:48:0.8 containing 18 mM sodium dodecyl sulfate
Column temperature: 40 ± 0.1
Flow rate: 2
Injection volume: 50
Detector: F ex 420 em 490

CHROMATOGRAM
Retention time: 14
Limit of quantitation: 5 ng/mL

KEY WORDS
derivatization; plasma; ultrafiltrate

REFERENCE
Sprancmanis,L.A.; Riley,C.M.; Stobaugh,J.F. Determination of the anticancer drug, 15-deoxyspergualin, in plasma ultrafiltrate by liquid chromatography and precolumn derivatization with naphthalene-2,3-dicarboxaldehyde/cyanide, *J.Pharm.Biomed.Anal.*, **1990**, *8*, 165–175.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. 750 μL Plasma + 30 μL 70% perchloric acid, vortex, centrifuge at 15600 g for 5 min, filter (0.22 μm) the supernatant, inject a 10-200 μL aliquot of the filtrate. Urine. Dilute urine 10 to 25-fold with water, centrifuge, filter (0.22 μm) the supernatant, inject a 10-200 μL aliquot of the filtrate.

HPLC VARIABLES
Guard column: 15 × 3.2 7 μm Newguard RP 18
Column: two 100 × 4.6 5 μm RP 18 columns in series (Brownlee)
Mobile phase: Gradient. A was MeCN:100 mM pH 2.55 NaH_2PO_4 containing 8 mM octanesulfonic acid and 0.1 mM EDTA 2:98. B was MeCN:200 mM pH 3.1 NaH_2PO_4 containing 8 mM octanesulfonic acid 30:70. A:B from 55:45 to 25:75 over 20 min.
Flow rate: 1
Injection volume: 10-200
Detector: F ex 340 em 440 following post-column reaction. The column effluent mixed with the reagent pumped at 0.7 mL/min and flowed through a 2 m long reaction coil at 41° to the detector. The reagent was 500 mM pH 8.8 potassium borate buffer containing 0.8 g/L o-phthaldialdehyde, 1% MeOH, and 0.06% 2-mercaptoethanol.

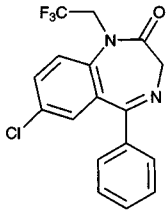
CHROMATOGRAM
Retention time: 22
Limit of quantitation: 100 nM

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
use plasticware; plasma; post-column reaction; pharmacokinetics

REFERENCE
Muindi,J.F.; Lee,S.-J.; Baltzer,L.; Jakubowski,A.; Scher,H.I.; Sprancmanis,L.A.; Riley,C.M.; Vander Velde,D.; Young,C.W. Clinical pharmacology of deoxyspergualin in patients with advanced cancer, *Cancer Res.*, **1991**, *51*, 3096–3101.

Halazepam



Molecular formula: $C_{17}H_{12}ClF_3N_2O$
Molecular weight: 352.74
CAS Registry No.: 23092-17-3
Merck Index: 4619

SAMPLE
Matrix: blood
Sample preparation: Condition a Bond-Elut C8 SPE cartridge with 2 mL MeOH and 2 mL water, do not allow to dry. Add 100 μL 5 ng/mL diazepam in 1 M pH 10.5 glycine buffer then 1 mL plasma to the SPE cartridge, wash with 2 mL water, wash with 50 μL MeOH, elute with three 200 μL aliquots of MeOH. Combine the eluates and evaporate them to dryness under a stream of nitrogen at 37°, reconstitute the residue in 100 μL mobile phase, inject a 50-80 μL aliquot.

HPLC VARIABLES
Column: 100 × 4.6 3 μm Adsorbosphere C8
Mobile phase: MeOH:20 mM pH 4.0 phosphate buffer 60:40
Flow rate: 1
Injection volume: 50-80
Detector: UV 240

CHROMATOGRAM
Retention time: 9.31
Internal standard: diazepam (7.76)
Limit of quantitation: 1 ng/mL

OTHER SUBSTANCES
Extracted: nordiazepam
Simultaneous: alprazolam, chlordiazepoxide, clonazepam, desmethyldiazepam, 3-hydroxyhalazepam, lorazepam, methylclonazepam, oxazepam, prazepam, quazepam, temazepam, triazolam

KEY WORDS
SPE; plasma; pharmacokinetics

REFERENCE
Gupta,S.K.; Ellinwood,E.H. Liquid chromatographic assay and pharmacokinetics of halazepam and its metabolite in humans, *J.Pharm.Sci.*, **1990**, *79*, 822–825.

SAMPLE
Matrix: microsomal incubations
Sample preparation: 2.5 mL Microsomal incubation + 2.5 mL acetone, add 30 μL diazepam in MeOH, add 2.5 mL chloroform, centrifuge. Remove the organic layer and evaporate it to dryness under reduced pressure at 40°, reconstitute the residue in 100 μL mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 250 × 6.2 7 μm Zorbax silica